

WHAT IS CLAIMED IS:

1. A purified protein having the ability to oxidize a retinoid, and having an amino acid sequence which is at least about 30% conserved in relation to the amino acid sequence identified as SEQ ID NO:2 or identified as SEQ ID NO:4 or identified as SEQ ID NO:32, or a functionally equivalent homolog thereof.
5. A said protein of claim 1, in which the protein is at least about 35% conserved in relation to the amino acid sequence identified as SEQ ID NO:2 or identified as SEQ ID NO:4 or identified as SEQ ID NO:32, or a functionally equivalent homolog thereof.
10. A said protein of claim 1, in which the protein is at least about 50% conserved in relation to the amino acid sequence identified as SEQ ID NO:2 or identified as SEQ ID NO:4 or identified as SEQ ID NO:32, or a functionally equivalent homolog thereof.
15. A protein according to claim 1, or a conservatively substituted variant thereof.
20. The protein of claim 1, wherein the retinoid is a retinol and/or a retinoic acid.
25. The protein of claim 1, wherein the protein has the ability to oxidize the carbon at the 4-position of the β -ionone ring of the retinoid.
30. The protein of claim 6, wherein the retinoid is an all-*trans* retinoid.
35. A purified protein having the ability to hydroxylate retinoic acid at the 4 position of the β -ionone ring of retinoic acid, and having an amino acid sequence which is at least about 30% conserved in relation to the amino acid sequence identified as SEQ ID NO:2 or identified as SEQ ID NO:4 or identified as SEQ ID NO:32, or a functionally equivalent homolog thereof.
40. A purified protein having the ability to oxidize a retinoid at the carbon in the 4-position of the β -ionone ring of the retinoid.
45. The protein of claim 9 wherein the retinoid is an all-*trans* retinoid.
50. The protein of claim 10 wherein the retinoid is a retinoic acid.
55. The protein of claim 1, or a conservatively substituted variant thereof.
60. An isolated nucleic acid molecule encoding a protein of claim 1, or a nucleic acid strand capable of hybridizing with the nucleic acid molecule under stringent hybridization conditions.
65. An isolated nucleic acid molecule encoding a protein of claim 8.
70. An isolated nucleic acid molecule comprising a DNA molecule having the sequence identified as SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:31, or which varies from the sequence due to the degeneracy of the genetic code, or a nucleic acid strand capable of hybridizing with at least one said nucleic acid molecule under stringent hybridization conditions.
75. A said nucleic acid molecule of claim 15 wherein the sequence of the nucleic acid molecule corresponds to a part of a human genome or of a fish genome or of a mouse genome, or varies therefrom due to the degeneracy of the genetic code.
80. Isolated mRNA transcribed from DNA having a sequence which corresponds to a nucleic acid molecule according to claim 15.
85. A microbial cell containing and expressing heterologous DNA encoding a retinoid inducible protein.
90. A microbial cell according to claim 18 in which the protein is capable of oxidizing a retinoid.

20. A microbial cell containing and expressing heterologous DNA encoding a retinoid inducible protein having all-trans retinoic acid 4-hydroxylase activity.

21. The cell of claim 18 wherein the retinoid is retinoic acid.

22. A microbial cell containing and expressing heterologous DNA which is complementary a nucleic acid molecule of claim 13.

23. Isolated DNA having a sequence according to claim 15 in a recombinant cloning vector.

24. A stably transfected cell line which expresses a protein according to claim 1.

25. A culture of cells transformed with a recombinant DNA molecule having a nucleic acid sequence according to claim 15.

26. A host cell that has been engineered genetically to produce a protein according to claim 1, the cell having incorporated expressibly therein heterologous DNA encoding said protein.

27. The cell of claim 26 wherein production of the protein is inducible by exposing the cell to retinoic acid.

28. The cell of claim 26 wherein the cell is eukaryotic.

29. A process for producing the protein of claim 1 comprising:
preparing a DNA fragment including a nucleotide sequence which encodes said protein;
incorporating the DNA fragment into an expression vector to obtain a recombinant DNA molecule which includes the DNA fragment and is capable of undergoing replication;
transforming a host cell with said recombinant DNA molecule to produce a transformant which can express said protein;
culturing the transformant to produce said protein; and
recovering said protein from resulting cultured mixture.

30. An antibody to a protein of claim 1.

31. An antibody of claim 30 wherein the antibody is a monoclonal antibody.

32. An antibody to a protein having a sequence included in the sequence identified as SEQ ID NO:4.

33. A protein of claim 1 for use in metabolizing retinoic acid in an organism or cell in need of such metabolizing.

34. A method for metabolizing retinoic acid in an organism or cell in need of retinoic acid metabolizing comprising administering a protein of claim 1.

35. A method for inhibiting retinoic acid hydroxylation in an organism in need of such inhibition, comprising administering to the organism an effective amount of an antisense nucleic acid or oligonucleotide substantially complementary to at least a portion of the sequence identified as SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:31.

36. The method of claim 35 wherein the portion is at least 5 bases in length.

37. The method of claim 35 wherein the organism is human.

38. The method of claim 35 wherein the organism is being treated for a disease selected from the group consisting of cancer, actinic keratosis, oral leukoplakia, a secondary tumor of the head and/or neck, a non-small cell lung carcinoma, a basal cell carcinoma, acute

promyelocytic leukemia, skin cancer, and a premalignancy associated actinic keratosis, acne, psoriasis and/or ichthyosis.

39. The method of claim 38 wherein the disease is acute promyelocytic leukemia.

40. A method for producing a desired protein, comprising:

- 5 providing a cell which can produce an endogenous protein in response to exposure to a retinoid;
- incorporating into DNA of the cell a DNA sequence encoding the desired protein at or near a site which is normally occupied by a DNA sequence encoding the endogenous protein; and

10 exposing the cell to the retinoid so as to induce production of the desired protein.

41. A kit for determining the presence of a protein according to claim 1 or having an amino acid sequence identified as SEQ ID NO:4, comprising an antibody to said protein linked to a reporter system, wherein the reporter system produces a detectable response when a predetermined amount of the protein and the antibody are bound together.

- 15 42. A kit for determining the presence of a first said nucleic acid molecule of claim 13, the kit comprising a second nucleic acid molecule capable of hybridizing with at least a portion of a the first nucleic acid molecule under stringent conditions, in which the second nucleic acid molecule is linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the first and second molecules are hybridized
- 20 together.

43. The kit of claim 42, wherein the nucleic acid molecule is at least about 5 bases in length.

44. An isolated DNA molecule having a nucleotide sequence selected from the group consisting of:

- (a) SEQ ID NO:33;
- 25 (b) SEQ ID NO:34;
- (c) SEQ ID NO:35; and
- (d) a fragment of (a), (b) or (c),

wherein said DNA molecule possesses promoter activity.

45. A DNA molecule of claim 44 wherein said DNA molecule includes the sequence

30 TGAAC(TN)_xTGAAC(T), wherein x has a value of up to 5.

46. A DNA molecule of claim 45 wherein x is 5.

47. A DNA molecule of claim 45 wherein said DNA molecule further includes the sequence TCTGASSAAGKTAAC downstream from the sequence TGAAC(TN)_xTGAAC(T).

48. A DNA molecule of claim 47 further including the sequence AATT between the sequence

35 TGAAC(TN)_xTGAAC(T) and the sequence TCTGASSAAGKTAAC.

49. A DNA molecule of claim 48 wherein there are up to six nucleotides between the sequence TGAAC(TN)_xTGAAC(T) and the sequence TCTGASSAAGKTAAC.

50. A DNA molecule of claim 45 further comprising the sequence CAATTAAAGA upstream of the sequence TGAAC(TN)_xTGAAC(T).

51. A DNA molecule of claim 44 wherein said DNA molecule includes the sequence CAATTAAAGATGAACCTTGGGTGAACTAATT.
52. A DNA molecule of claim 44, 45, 46, 47, 48, 49, 50 or 51 wherein the said DNA molecule includes the sequence TATAA.
- 5 53 A DNA molecule of claim 45, 46 or 50, wherein the said DNA molecule includes the sequence TATAA downstream of the sequence TGAAC(TN)xTGAAC.
54. A DNA molecule of claim 47 or 48, wherein the said DNA molecule includes the sequence TATAA downstream of the sequence TCTGASSAAGKTAAC.
- 10 55. A DNA molecule of claim 45 wherein said DNA molecule includes the sequence TATAA located downstream of the sequence TGAAC(TN)xTGAAC and spaced up to about 55 nucleotides therefrom.
56. A recombinant DNA comprising the isolated DNA molecule according to claim 44.
57. A recombinant DNA according to claim 51, further comprising one or more structural genes.
- 15 58. A recombinant DNA according to claim 56, wherein the one or more structural genes encodes cytochrome P450 protein(s).
59. An expression plasmid comprising the recombinant DNA according to claim 56.
60. An isolated cell containing the recombinant DNA according to claim 57.
61. An isolated cell according to claim 56 wherein said cell is eukaryotic.
- 20 62. A process for the production of recombinant protein(s), comprising culturing a cell according to claim 60 and recovering the protein(s) produced.
63. A process according to claim 62 wherein the protein(s) comprise a cytochrome P450.
64. A process according to claim 62 wherein the protein(s) comprise a fusion protein.
65. A method of screening drugs for their effect on activity of a retinoic acid inducible protein
- 25 comprising exposing a purified said protein to a said drug and determining the effect on the activity.
66. The method of claim 65 wherein the activity is hydroxylation of a retinoic acid, particularly all-trans retinoic acid.
67. A method of screening drugs for their effect on activity of a protein of claim 1 comprising exposing a purified said protein to a said drug and determining the effect on the activity.
- 30 68. The method of claim 67 wherein said method includes ascertaining the effect a said drug has on the oxidation of retinoic acid.
69. The method of claim 68 wherein said retinoid is all-trans retinoic acid.
70. A method of screening drugs for their effect on expression of a gene wherein the gene is
- 35 inducible by a retinoid, comprising exposing a recombinant DNA of claim 60 to a said drug and determining the effect on gene expression.
71. The method of claim 70 including exposing the recombinant DNA in the presence of a said retinoid.
72. The method of claim 70 wherein said structural gene is a reporter gene which does not
- 40 metabolize retinoic acid.

73. The method of claim 70 wherein determining the effect on gene expression includes ascertaining the effect on transcription of the gene.
74. A method of screening a drug for its effect on the metabolism of a retinoid by a cytochrome P450 encoded by a nucleotide sequence identified according to claim 1 and incorporated into 5 an expression system so as to be under control of a nucleotide sequence having native promoter activity for a said cytochrome P450, comprising:
exposing the system to the drug in the presence of the retinoid so as to determine the effect of the drug on metabolism of the retinoid.
75. The method of claim 74 wherein the retinoid is retinoic acid.
- 10 76. The method of claim 74 or claim 75 wherein the retinoic acid is all *trans*-retinoic acid.
77. A drug identified according to a method of claim 65, 66, 67, 68 or 69 as having the effect of modulating the activity of a said protein.
78. A drug identified according to a method of claim 70, 71, 72, 73, 74, or 75 as having the effect of modulating gene expression.
- 15 79. A drug identified according to a method of claim 76 as having the effect of modulating gene expression.
80. A method for inhibiting retinoic acid metabolism in an organism in need of such inhibition, comprising administering to the organism an effective amount of a drug of claim 77.
81. A method for inhibiting retinoic acid metabolism in an organism in need of such inhibition, 20 comprising administering to the organism an effective amount of a drug of claim 78.
82. A method for inhibiting retinoic acid metabolism in an organism in need of such inhibition, comprising administering to the organism an effective amount of a drug of claim 79.

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